EFFECTS OF GENETIC AND ENVIRONMENTAL VARIABLES ON RESPONSIVENESS TO IMIPRAMINE IN RATS

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CONTENTS

ABSTRACT

Preferences for novelty, ambulation, rearing and grooming were observed in isolated and grouped rats of both sexes, following chronic administration of saline, 10mg/kg or 20mg/kg of imipramine. Sex differences were found on preferences for novelty, with responses varying according to the dosage level of the drug and the time period within which the observation was made. Drug suppression of ambulation, rearing and grooming was evident in both sexes, and this was suggested to be related to the sedative properties of imipramine. The initial hypotheses that social isolation may be useful as an animal model of psychopathology in antidepressant drug research was not strongly supported. However, it was concluded that a more thorough investigation of this and other environmental manipulations is warranted. It was furtherconcluded that the sex of the subjects should be taken into account when considering the effect of imipramine on higher CNS mechanisms, and also in the therapeutic use of imipramine.

CHAPTER

INTRODUCTION

The rapid and widespread acceptance of psychotropic drugs into clinical practice over the past twenty years has had a profound effect on the treatment of mental disturbances. However, it seems that this acceptance, generally, and in relation to the antidepressant drug, imipramine, with which this research is concerned, has not been accompanied by a sound understanding of the basic mechanisms of action of such drugs. It is becoming increasingly clear that the continued improvement of the therapeutic application of psychotropic compounds demands an understanding of these mechanisms.

Experimentation at an animal level is a vital part of this process of understanding. However, a review of the literaure in the area reveals that beyond mere toxicological screening procedures, insufficient attempts have been made to analyse the highly complex relationship between drug action and behaviour at this basic level.

As in most areas of animal research, evaluation of the reported effects of drugs on animal behaviour is made difficult by the wide variety of experimental and organismic variables involved. The literature comprises studies using different dosage levels of the drugs, administered by different routes,

and for varying lengths of time. Frequently, inadequate consideration has been given to the importance of genetic factors such as species, strain and sex differences, as well as to the effect of prior experiences. Of particular relevance in the study of antidepressant drugs is that effects have sometimes been determined on normal animals, on other occasions on animals with an experimentally-induced "depression". To add to the complexity of the situation, behavioural assessments have been carried out in a wide variety of test situations thus making a comparison of results difficult. All of these are important but frequently disregarded factors in the construction of animal research studies. It is hoped that the present "exploratory" research will contribute to the understanding of the complexities involved in this area by focussing on the importance of some of these variables, with particular reference to imipramine.

Imipramine

Imipramine (IMI), which belongs to the tricyclic group of antidepressant drugs, was introduced by Kuhn in 1957. Its antidepressant properties were discovered clinically rather than pharmacologically, during investigations of its potential use as a tranquilliser. Numerous investigations of IMI have verified that it is an effective agent for the treatment of depression. According to Angst (1970) IMI is "still the standard drug for the treatment of depression ... and has been more thoroughly investigated than any other antidepressant."

Pharmacologically, IMI has many properties similar to

chlorpromazine, a tranquiliser of the phenothiazine group.

Biochemically, IMI has been shown to inhibit the uptake of norepinephrine in the rat brain, and to alter its turn-over and metabolism. It has thus been suggested that changes in the biogenic amine metabolism produced by the tricyclic antidepressants may account for their clinical effects.

In human depressed patients the therapeutic effect of IMI is not immediate, and is generally manifest from one to three weeks after the commencement of treatment. IMI appears to have little effect on normal human subjects, other than a sedative effect, which is also frequently associated with its clinical effect.

Pharmacological variables of Importance in Drug Research

1. Dosage Levels

Although it is generally appreciated in pharmacological research that responses will vary according to the dosage level of the drug used, no clear understanding of this relationship appears to exist in relation to IMI.

In most experiments on normal animals low doses of IMI (less than 10mg/kg) have no significant effect. An increase in the dose usually results in a sedating action, with sub-toxic doses provoking excitation, followed by convulsions.

These types of effects correspond in general to those observed in man i.e. in normal test subjects and in cases of poisoning. Inconsistencies, however exist, with the finding in certain experiments of a stimulant response to low doses of IMI. It must be pointed out however that these findings apply to normal animals, and may have little relation to its therapeutic use.

2. Duration of Drug Administration

Although, as previously mentioned, the tricyclic antidepressants require long-term administration for the achievement of a therapeutic effect in depressed patients, most animal experimentation with IMI has been performed after a single administration of the drug. This suggests that much of the research carried out on animal subjects may contribute little to the understanding of its clinical action.

A number of recent reports provide evidence that differences do exist between the effects of acute and chronic administration of IMI on animal behaviour. The effects have been observed biochemically as well as behaviourally.

Schildkraut, Winokur and Applegate (1970) found differences in the uptake, turnover and metabolism of norepinephrine, a catecholamine frequently implicated in the etiology of depression, after single and repeated doses of IMI.

From a behavioural point of view, Furgiuele, Aument and

Horovitz (1964) showed that chronic IMI treatment over a period of twenty six days reduced the spontaneous activity of normal rats and rats with lesioned amygdalae. Similar findings were reported by Meltzer and Fox (1971) as the immediate effects of IMI after intermittent administration. However, an increase in spontaneous activity was observed one day after IMI administration. Kulkarni and Dandiya (1973) investigated the effects of acute and chronic administration of tricyclic compounds on activity in an open field, in which the frequency of ambulation and rearing responses were observed. Ambulation was reduced after a single dose, which was consistent with the previous observations. Chronic administration however, selectively increased the rearing response, with a concomitant reduction in ambulation.

These few studies highlight the difficulties in constructing a meaningful picture of the effects of IMI on animal behaviour. As Taber (1971) points out, it is difficult to design chronic experiments in the absence of complete dose and time response information. He suggests that "more chronic experimentation could give important insights into the ways that drugs interact with biochemical, neurophysiological and behavioural levels of integration, during regimens more relevant to the clinical situation."

Search for an Adequate Animal Model of Depression

It has already been mentioned that pharmacological drug research utilising normal subjects may provide little information on the nature of the effect of antidepressive drugs. Thus it is important that investigations be made in the context of a model system that resembles the pathological condition found in man. Unfortunately, a valid animal model of depression does not yet exist.

One factor in this situation is the confusion and lack of specificity surrounding the term "depression". As Lehmann (1959) points out, "the term may refer to a symptom, a syndrome or a nosological entity." Although definitions of depression include observable behavioural patterns such as psychomotor retardation, anorexia, weight loss and sleep disturbances, the fundamental feature in man consists of a change in mood, a phenomenon which is impossible to evaluate in other animals. Thus in the absence of "grief-stricken" or endogenously depressed rats, researchers have relied on pharmacological models of depression. The most widely used model is the reserpine model of depression, which has largely been based on the catecholamine hypothesis for mood suggested by Schildkraut (1965) and discussed at length by subsequent investigators.

Initially it was intended to use the reserpine model in this research, but a perusal of the recent literature throws into serious doubt the validity of such a procedure. Because

of its widespread acceptance as a valid model it seems important to discuss some of these more recent findings, and thus the rationale for its rejection in this research.

Reserpine Model of Depression

The use of the reserpine model of depression was based originally on the observation that a consistent number of humans receiving reserpine treatment for hypertension developed severe depressive conditions. It was also observed that animal subjects developed a psychomotor retardation which was suggested to be related to the human response to the drug, and which could be reversed by both types of antidepressant drugs effective in the treatment of depression i.e. the monoamine oxidase inhibitors, and the tricyclic compounds.

The behavioural findings seemed to be supported by the biochemical findings in animals. It was found that reserpine caused a depletion in the catecholamines norepinephrine and dopamine, whereas the antidepressant drugs increased these amines. It was thus suggested that a deficiency of the catecholamines was of major etiological significance in depression. Other researchers provided evidence that the indoleamine serotonin may be the important amine involved. (Pletscher, Shore and Brodie, 1956; Richter, 1967; Glassman, 1969)

However, the significance of these biochemical events implicated from animal research remains to be elucidated in the

human condition of depression. At present, any investigations of the relationship of the biogenic amines to depression must be indirect, mainly focussing on findings from urine, blood and cerebrospinal fluid from depressed patients. It is extremely difficult to study directly the metabolism of amines in the brains of depressive patients, and studies of peripheral amine activity are of doubtful validity.

Although biochemical processes are likely to be involved in the etiology of depression, the amine hypotheses appear to be an oversimplification of a higher complex phenomenon. Thus from the point of view of its strong reliance on these biochemical hypotheses, the reserpine model does not seem adequate.

Further, a recent report by Mendels and Frazer (1974) suggests that, in man, reserpine precipitates depression in a relatively small number of susceptible persons, rather than causing the syndrome. Reports of reserpine-induced depression during the 1950's and 1960's claimed an incidence of 15 per cent amongst patients receiving the drug for other conditions. However, many of the reports are retrospective and poorly documented, and it seems that those who do develop depressive symptoms in response to reserpine are likely to have had a past history of depression. It is also suggested that rather than depression, reserpine produces primarily psychomotor retardation in man, with presumably a similar effect in animals, rather than an alteration of some higher mood process.

In view of the tenous assumptions on which the reserpine model of depression rests, and the narrow focus of past antidepressant drug research on this, it seems essential that other test procedures, based not on drug interactions, but rather on modes of behaviour wherein depression is induced by environmental factors, be investigated.

Social Isolation as a Model of Depression

As loss or lack of social interactions appears to be important in the development of some human depressions, it was decided that manipulation of the social environment of animals be investigated for its potential use in antidepressant drug research.

Behavioural abnormalities resulting from the manipulation of social variables are well known in the context of separation experiments carried out on non-human primates (e.g. Harlow, 1959; Harlow and Zimmerman, 1959; Seay and Harlow, 1965), and from observations of human infants separated from their mothers (e.g. Spitz, 1946; Robertson and Bowlby, 1952). In both cases, separations have caused emotional disturbances, often enduring, that have been described as similar to depressive reactions in adults.

In addition to separation, social isolation has potential as a method of inducing "depression" in animals. Social

isolation in monkeys has been found to induce severe emotional disturbances, primarily involving fear responses. McKinney and Bunney (1969) suggest that "the age at which social isolation is imposed and the duration of its imposition, may be relevant in determining the nature of the response induced. Perhaps at certain developmental stages the reaction would be 'depression' rather than fear."

The effects of social isolation have been widely investigated in rodent subjects, and with respect to behaviours interpreted as measures of "emotionality"in the rat, it has been fairly consistently reported that animals reared in isolation are more emotional than group-reared animals.

Meyers and Fox (1963) found that when tested on a "five choice point multiple U maze", isolation-reared animals scored significantly higher than group-reared animals on numbers of errors and number of trials to reach a criterion of ten consecutive errorless trials. The authors attributed their findings to reduced exploratory behaviour and more emotional responses amongst the isolation-reared animals. Hahn (1965) reached similar conclusions from results of an "emergence-test", recording running latencies to a reward of wet mash along a runway. Isolated animals had longer latencies, which Hahn attributed to more "timidity" than the grouped animals. Moyer and Korn (1965) found that isolates were more emotional as measured by startle responses to a loud noise. Archer (1969) isolated female rats for 24 weeks and found that this led to

lower activity levels than animals housed in groups of three, five and eight. No significant effect was found amongst males.

Although the concept of emotionality in animals is a controversial one, it is used here in a broad sense, as a useful framework from which to work. Since the controversy, as discussed by Archer (1973), centres on the inadequacy of the measures used in relation to emotionality (mainly the activity measures), the usefulness of isolation-induced emotionality will be evaluated largely in relation to non-activity measures.

A differential responsiveness to psychotropic drugs of group-reared and isolation- reared animals has been reported. Baumel, de Feo and Lal (1969) found that social isolation reduced the duration of hypnosis due to short-acting barbiturates. Hughes and Syme (1972) reported differences with chlordiazepoxide and methlyphenidate. Isolated rats ambulated more after both 3.75mg/kg and 5.0mg/kg of chlordiazepoxide, whereas an inverted U relationship between dose strength and ambulation was apparent with group-reared animals. Under methylphenidate there was no difference between grouped and isolated animals on ambulation, but the latter showed significantly fewer rearing responses.

Support for the suggestion that social isolation may be a useful means of manipulating the emotional level of animals for the investigation of antidepressant drugs comes from a study by Valzelli and Bernasconi (1971). They found that the tricyclic antidepressants, IMI and desipramine, blocked the muricide reaction in rats completely and for a long time.

The muricide reaction has been reported on a number of occasions as one reaction produced by prolonged social isolation.

Sex Differences in Response to Psychotropic Drugs

The importance of the sex variable in psychological research is a commonly disregarded factor. According to Harris (1972), much of the literature apparently deals with males and females only as an "undifferentiated mass", or else tends to employ male subjects to the exclusion of female subjects.

In research on affective disorders and the effects of psychotropic drugs it seems of great importance to evaluate effects on both males and females, since sex differences exist in biochemical reactivity and metabolic and endocrine functioning, systems which are likely to be important in affective disorders.

It has also been reported that twice as many men as women are treated for depression, and the literature suggests a differential responsiveness to antidepressant drugs, with men benefitting more than women. (Raskin, 1974).

Sex differences in response to psychotropic drugs have been noted with animal subjects. Irwin, Slabok and Thomas (1958) found females to be more sensitive than males to stimulant or depressant drugs on tests of locomotor activity. However, in a conditioned avoidance procedure, females were found

to be more resistant to drugs suppressing learning of the avoidance response than were males.

The role of the sex variable in determining drug effects has been recognised in several studies investigating various drug effects on exploratory behaviour. For example, Hughes and Syme (1972) found that the sex of the animals determined the effects of both chlordiazepoxide and methylphenidate on rearing responses, with an initial superiority of females being eliminated after administration of both drugs. Chlordiazepoxide also reduced preferences for novelty in male but not female subjects.

It is thus regarded as being of considerable importance to include the sex of the subjects as a variable in the design of this research on the effects of IMI.

Exploration as the Dependent Variable

Tests of exploratory behaviour are frequently used in the evaluation of psychotropic drug effects. Difficulties arise, however, because of the lack of theoretical agreement as to what constitues exploratory behaviour, the mechanisms underlying it, and by what means it can validly be measured.

In order to gain a perspective on the difficulties involved, the central points concerned will be discussed.

Although no single adequate definition of exploration

exists, a commonly accepted feature of the phenomenon is that "the basic determinants of exploration occur in the organism's external rather than internal environment." (Fowler, 1965). The important aspects of the external environment in the control of exploratory behaviour are defined by Berlyne (1960) as being the "collative properties such as novelty and complexity." For exploration to occur in response to this environmental stimulation, attentional and motivational processes within the organism must also be involved.

As motivational processes are presumed to be involved in psychopathological states such as depression, exploration, in terms of these conceptual features, would appear to be valid means of assesing psychotropic drug effects. Unfortunately, however, tests of exploratory behaviour have frequently not been adequately related to these features presumed to be important in exploration.

Probably the most frequently used measure of exploration has been ambulation in a novel environment. In fact, in many cases ambulation and exploration have been regarded as synonymous, with high ambulation reflecting high levels of exploratory behaviour. This has also been related to emotionality concepts, with high ambulation presumed to be related to low emotionality. These assumptions are naive in that it seems obvious that, for example, high activity scores may just as easily reflect high levels of escape behaviour related to high emotionality as low emotionality related to high exploratory behaviour.

Thus the use of ambulation alone as a measure of exploration is misleading. Because of the difficulty in distinguishing between its internally and environmentally-aroused components, it seems more valid to consider ambulation as a measure of general, non-specific activity, (presumably controlled by both internal and external factors, to varying degrees), rather than as an environmentally-elicited exploratory activity.

Similarly, the use of the rearing response as a measure of exploration is open to question. There is disagreement as to whether this activity is indicative of exploratory tendencies, emotionality or merely CNS excitability (Lat, 1963; Soubrié and Boissier, 1972; Archer, 1973). Thus rearing can only be regarded as a general activity measure, rather than as an index of exploration.

Because of the dissatisfaction with the validity of ambulation and rearing responses, some studies have focussed on procedures which measure more clearly an animal's responsiveness to novel aspects of its environment. (Ahtee and Shillito, 1970; Hughes and Dyne, 1970 and others). The method described by Hughes (1968), and used in the present study, allows for the measurement of approach to a novel environment from familiar surroundings. Thus from the amount of time spent in the novel as compared to the familiar area, a measure of the animal's responsiveness to novelty is obtained. "Although by no means satisfactory, exploratory indices involving reactions to novelty or complexity may be less contaminated by influences

arising from sources other than the external environment." (Hughes, 1973)

The Effects of Psychotropic Drugs on Exploration and Activity Measures.

A number of studies report that several different psychotropic compounds have the effect of decreasing preferences for novelty (i.e. exploration).

This was found using chlordiazepoxide (Ahtee and Shillito, 1970; Hughes, 1972; Hughes and Syme, 1972; Hughes and Greig, 1975), methylphenidate (Dyne and Hughes, 1970; Hughes and Syme, 1972) and scopolamine (Hughes, Blampied and Steward, 1975).

This decrease in preference for novelty was also reported for IMI by Cox and Tye (1975).

Varying results have been reported when activity measures have been used in the evaluation of these drugs. As mentioned previously, the general findings in relation to the effect of IMI on activity levels suggest a reduction in activity with increasing doses above 10mg/kg. This effect was also found by Cox and Tye (1975) with 5mg/kg and 20mg/kg IMI.

Perspective on the Present Study.

This research may be viewed as an exploration of the interaction of pharmacological, genetic and environmental variables in

rodent subjects. The effects of the antidepressant drug imipramine on exploratory behaviour are investigated with particular reference to sex differences in responsiveness, and in a model system of relevance to the clinical use of imipramine. Thus chronic administration of the drug is to be used, and the investigation carried out on animals with an experimentally-induced pathology. A major concern of the research is the investigation of the validity of social isolation-induced abnormality as an animal model of depression.

CHAPTER 2

METHOD

Subjects

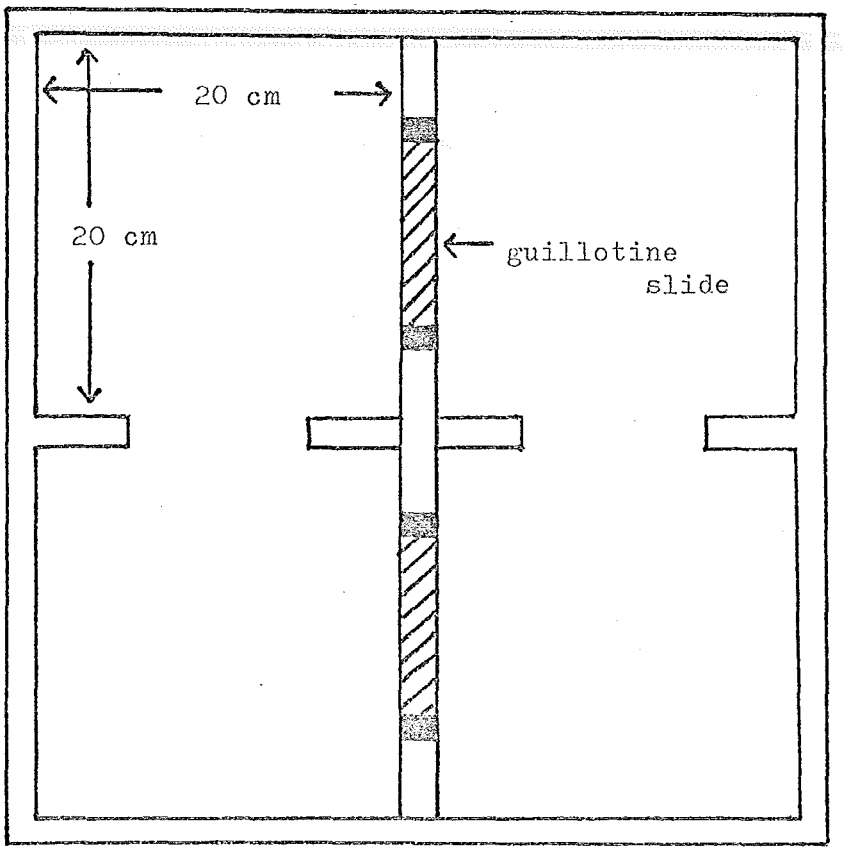
The subjects were thirty six male and thirty six female New Zealand black and white hooded rats. At weaning (25 days old) equal numbers of males and females were assigned to one of two conidtions. Animals in the "Isolated" condition were housed individually in cages 17 x 17 x 20 cm. In the "Grouped" condition animals were housed in cages 30 x 35 x 50 cm in groups of 6 animals of the same sex per cage.

Both types of cages were made of metal and had wood shavings on the floor. Food and water were freely available at all times. Temperature was kept constant at approximately 20°C and a reversed light-dark schedule was used.

Apparatus

The testing apparatus consisted of four perspex exploration boxes (see Figure A) of the design described in previous reports (e.g. Hughes 1968, 1972; Hughes and Swanberg, 1970). Each box consisted of four 20 x 20 x 20cm cells and could be divided into two similar halves of two cells each, by inserting opaque guillotine slides into two gaps in a perspex wall. The

Figure A



Floor Plan of the Exploration Box with Guillotine slides in Place.

boxes were covered with hinged transparent lids. The floor of each box consisted of a white painted steel grid and the entire apparatus was situated on a white tin tray. Each box was enclosed in a soundproof ventilated chamber and all observations were made through a one-way window 18 x 12cm in the front wall of the chamber. In this way the animals could be observed with the experimenter remaining obscured from view.

The testing area was illuminated by an 8 watt fluorescent lamp situated 24cm above each box. Auditory masking was provided by the noise of the extract fan in each chamber.

An electric timer was used to signal every fifth second.

Procedure

At approximately 100 days of age the rats were assigned to three drug treatment conditions. These were isotonic saline, 10mg/kg IMI and 20mg/kg IMI. Each treatment condition comprosed equal numbers of males and females, and isolated and grouped animals. There were thus three groups consisting of:

6 isolated males

6 isolated females

6 grouped males

6 grouped females

All rats received daily intraperitoneal injections for

fourteen days prior to testing. All injections were administered in a volume of 2mg/kg. The injecting procedure involved removal of the rat from its cage to a nearby table, where weighing was carried out prior to the administration of the appropriate drug level. Following this, the animal was returned to its home cage and left undisturbed until the next daily injection.

On the fifteenth day after the beginning of the chronic drug administration the exploration testing was carried out. Each rat was taken individually and placed for one hour in one half (the "familiar" half) of the exploration box, with the guillotine slides in place to prevent movement into the other half of the box. After one hour the rat was removed from the box. It was weighed and then injected with the level of drug appropriate to the treatment group to which it belonged i.e. with the same drug level it had received during the fourteen days of pre-testing drug administration. Following this, the animal was kept in a small individual cage for thirty minutes, to allow for the drug to take its effect. It was then replaced in the familiar half of the exploration box, from which the slides separating the two halves had been removed. The animal thus had access to both the "familiar" and previously inaccessible "novel" half.

Twenty seconds later, it was observed for a ten minute period, during which time it was noted every five seconds whether or not the animal was in the novel half of the

apparatus (preference for novelty), and whether it was engaging in rearing or grooming behaviours. Rearing was defined as "standing on hind legs either unsupported, or with front paws against the wall." Grooming was defined as "licking, scratching, rubbing or biting the body". (Hughes and Swanberg, 1970)

In addition, the number of cells entered was recorded by means of a hand counter.

CHAPTER 3

RESULTS

Five measures were obtained. These were preference for novelty, ambulation, rearing, grooming and body weight. Separate analyses of variance were performed on each measure. The first three measures fitted a 2 x 2 x 3 x 3 factorial design with repeated measures on the last factor. (c.f. Winer 1962). On these measures time was included as a factor. Thus by dividing the 10 minute observation period into three consecutive 200 second periods, 3 measures of this factor were obtained.

Because of the low overall response rate for grooming, total scores for the ten minute period were used. Thus a 2 x 2 x 2 factorial design was used for this measure.

An analysis of body weight was also carried out using the weights obtained on the first day of the chronic drug administration and the fifteenth day i.e. on the day of testing. A 2 x 2 x 3 x 2 design with repeated measures on the last factor was used.

Summaries of the analyses are given in the appendix. Results and graphs of all significant effects are presented

below. The level of significance used was $p < .05$.

Preference for Novelty

A significant 3 way interaction between sex, dose strength and time was obtained on preference for novelty.

As can be seen from Figure I this largely resulted from differential responding of males and females under the three levels of drug treatment during the third of the consecutive 200 second time periods.

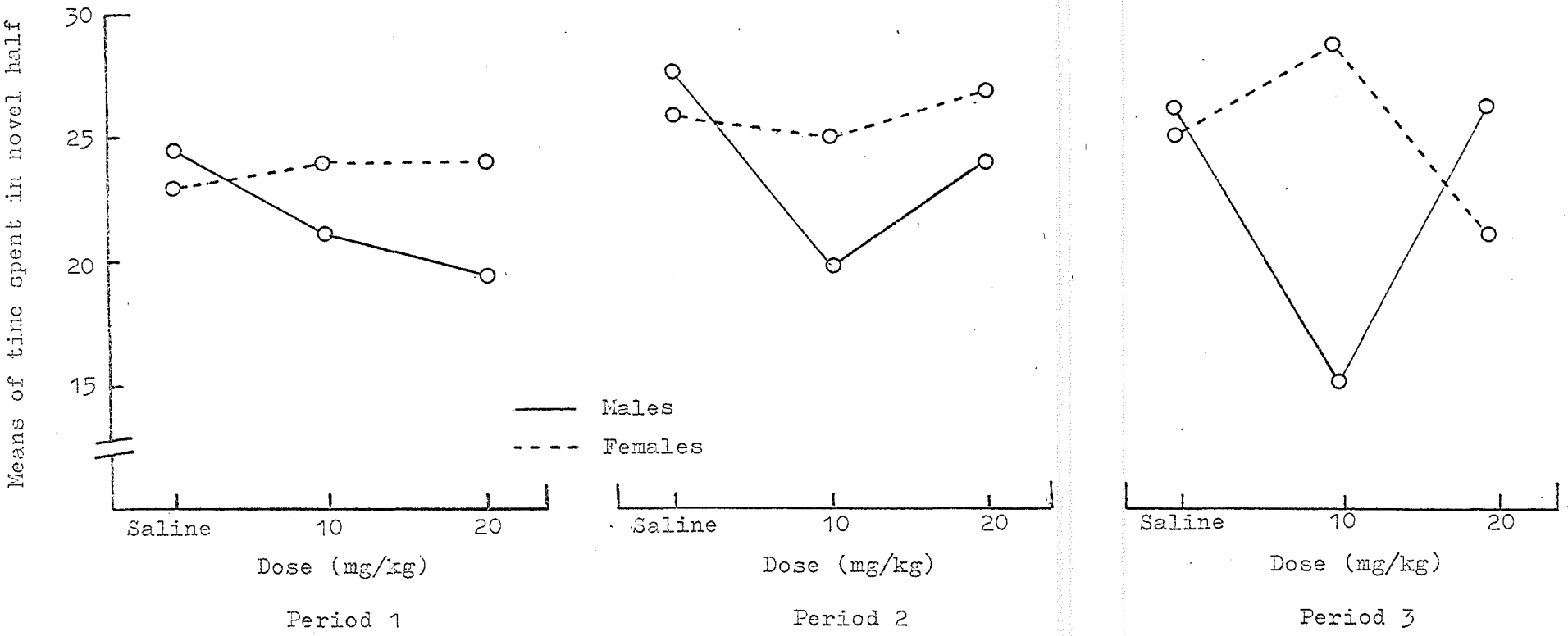
In the first time period both sexes showed similar response frequencies under the saline condition. A slight decrease was found for males with increasing dose strength, with the reverse trend for females.

In the second time period a decrease under 10mg/kg IMI, followed by an increase under 20mg/kg in novelty preferences was obtained by both males and females, though the effect was more marked for the former.

The results in these two time periods suggest only minor sex and dose strength related effects. The effects were most marked in the third period. As in the other time periods there was little difference between the sexes under the saline condition. Amongst males there was a marked decrease in novelty preference with 10mg/kg IMI followed by

Figure I

Preference for Novelty Scores



an increase with 20mg/kg, a pattern which was similar to, but more marked than that obtained for both males and females in the second time period. However, a reverse trend was apparent amongst females in the third time period, with an increase in preference for novelty under 10mg/kg as compared to saline animals, and then a decrease under 20mg/kg.

Ambulation

Significant drug and time effects were obtained on ambulation, as measured by the number of cells entered.

Figures II(a) and II(b) show that a reduction in ambulation occurred with increasing dose strength, and over consecutive time periods.

Also, an interaction effect between the sex and isolation variables approached significance. This suggests that, when reared in groups, females tended to ambulate more than males, but when reared in isolation this sex difference was not apparent.

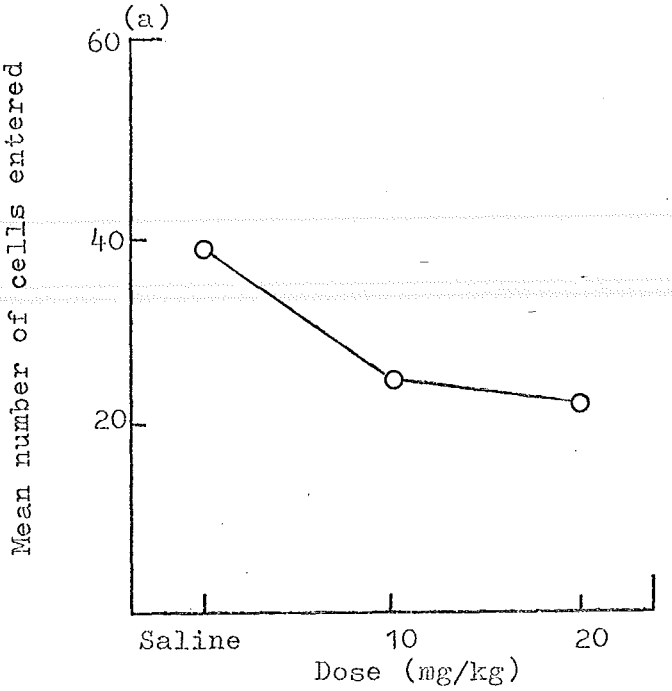
Rearing

On this measure significant effects were found for sex, dose strength and time variables. As seen in Figure III(a) females reared significantly more than males.

As with ambulation, rearing responses decreased with

Figure II

Ambulation Scores



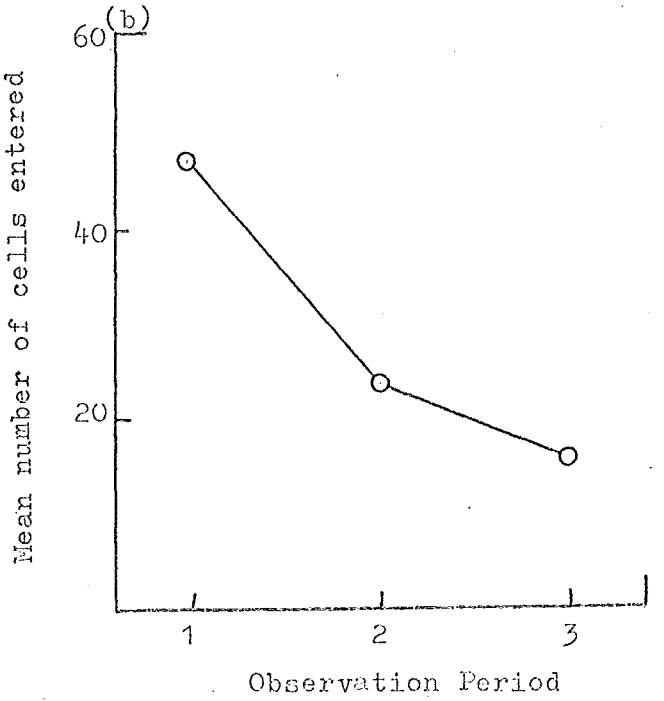
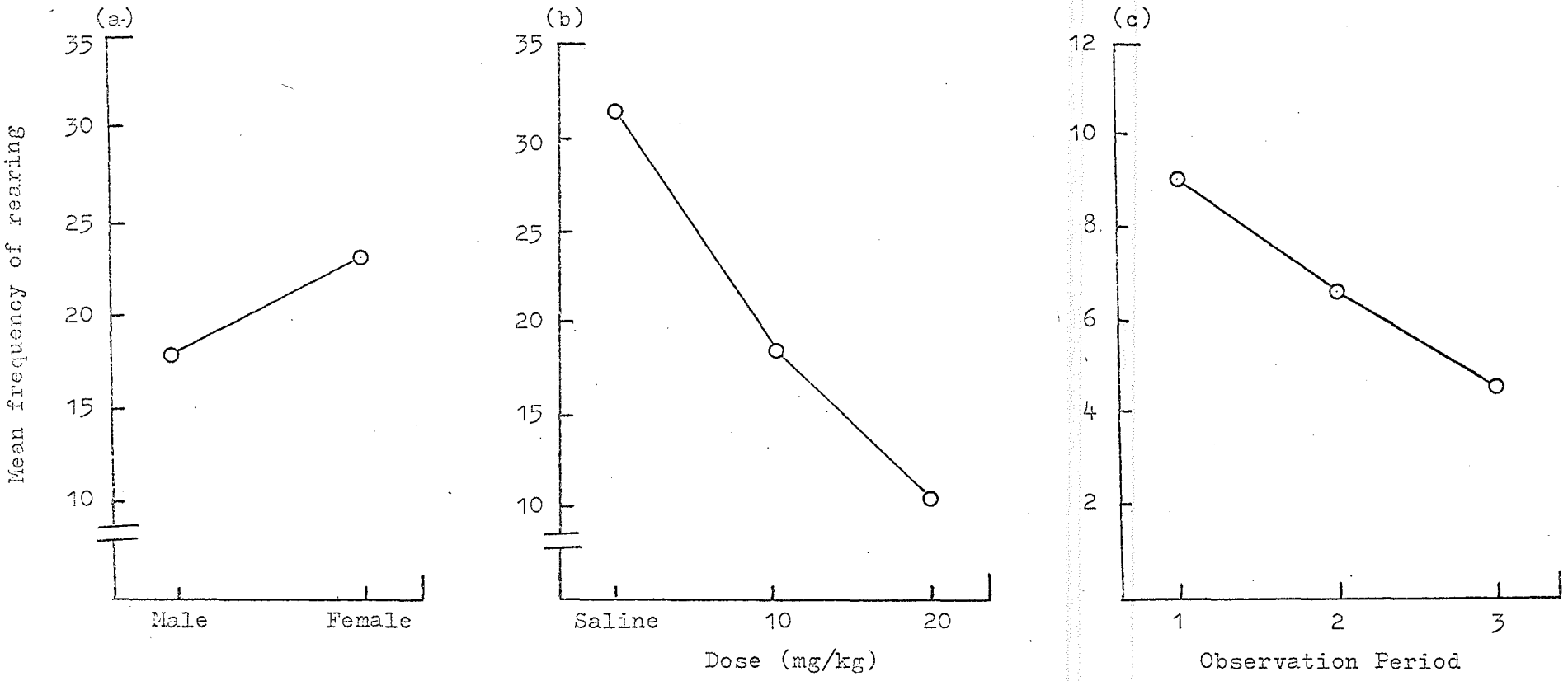


Figure III

Rearing Scores



increasing dose strenghts of IMI (Figure IIIb), and over the three consecutive time periods (Figure IIIc).

The effect of isolation on rearing approached significance, with isolated rats tending to rear less frequently than grouped rats.

Grooming

A significant two way interaction between isolation and dose strength was obtained on this measure.

Figure IV shows that, in the saline condition, isolated animals groomed significantly more frequently than grouped animals. Amongst isolated animals a steady decrease in grooming occurred as the dose strength increased. However, amongst group-reared animals grooming was not greatly affected by either dosage level of IMI. Therefore, dose strength had the effect of reducing the initially higher frequency of grooming amongst isolated animals to a level not significantly different from that of the grouped animals.

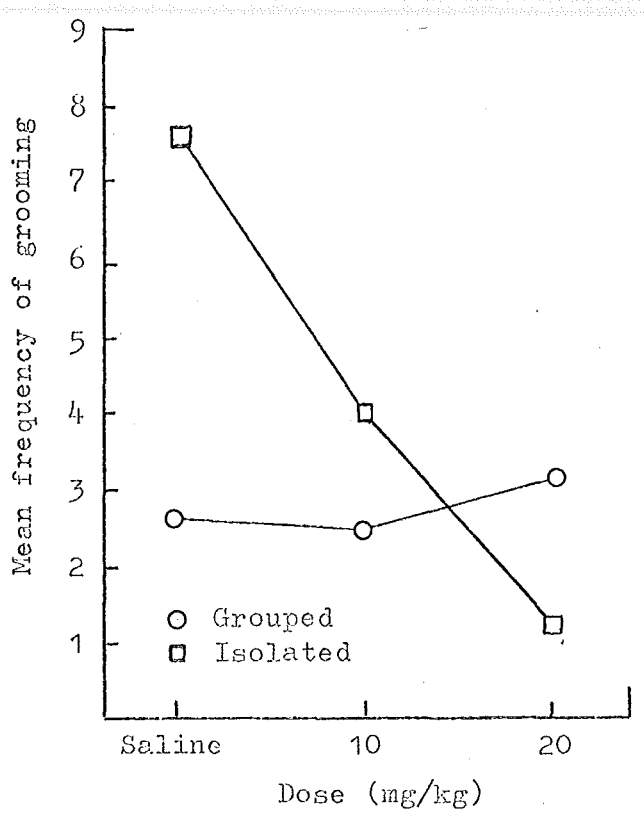
Body Weight

The effect of the chronic drug administration on body weight was manifested in a complex interaction of the three main variables.

A significant interaction between the sex and isolation

Figure IV

Grooming Scores



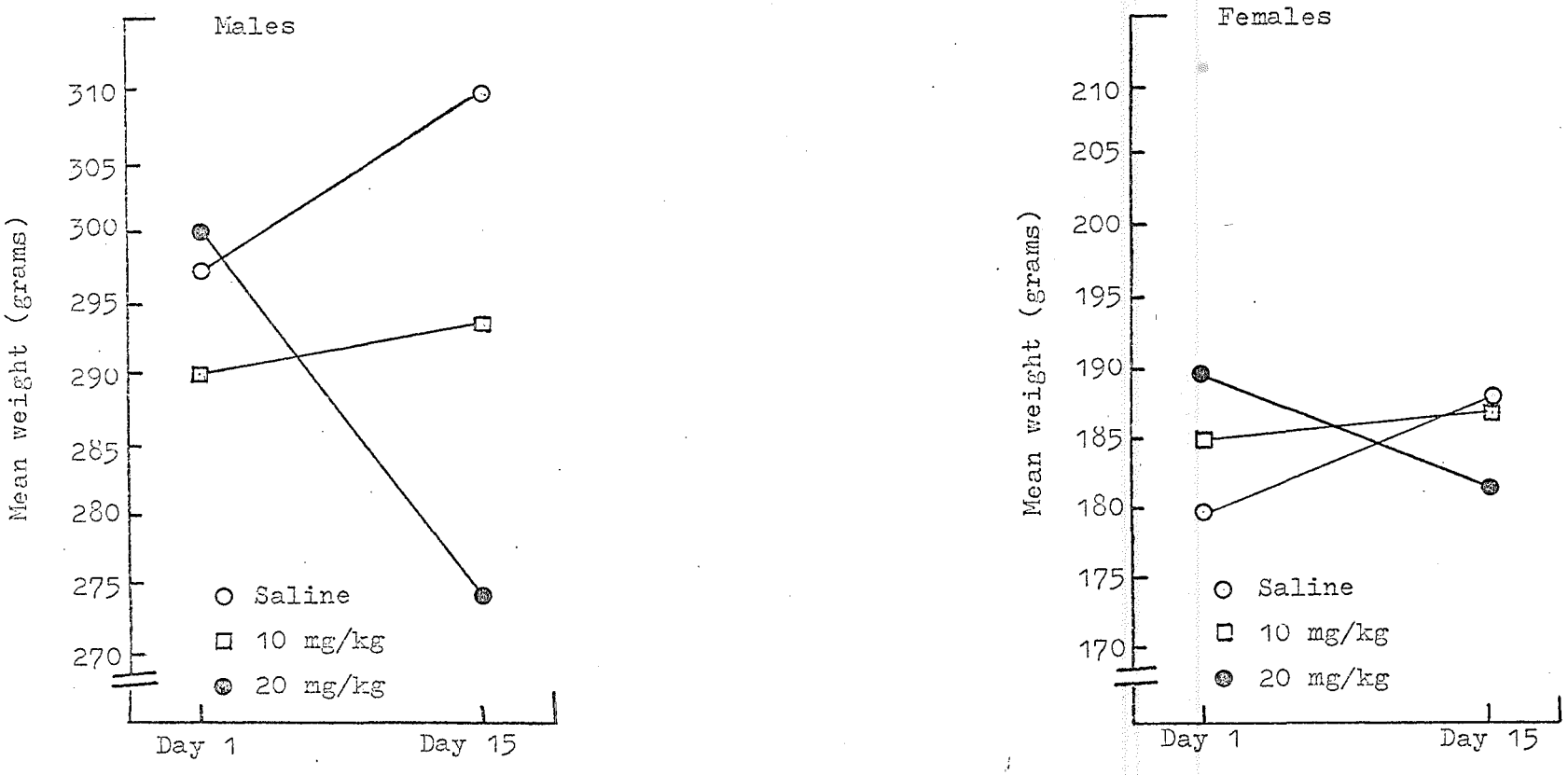
variables is shown in Figure V(a). From this it can be seen that, before drug treatment, isolated animals were heavier than grouped animals, amongst both males and females. Amongst male subjects, isolates showed a considerable decrease in weight over the drug treatment period, while grouped rats on the other hand showed little change. This had the effect of reducing the initial weight differences between the two groups. Amongst female subjects there was no significant change in weight during the treatment period, with both isolated and grouped animals remaining at a stable weight, and thus maintaining the initial superiority of isolated animals on this measure.

Figure V(b) indicates a significant interaction between the sex and dose strength variables. At the first weighing, when males and females are considered separately, animals in the three drug conditions were of approximately the same weight. This would be expected from the random assignment of subjects to the treatment groups. Over the drug treatment period, amongst males, the rats in the saline condition increased in weight, which would be expected from the fact that the animals were young and thus still growing. 10mg/kg IMI inhibited this weight gain, resulting in virtually no change over time. 20mg/kg IMI not only inhibited the expected weight gain, but was associated with a marked decrease in weight over the treatment period. A similar trend was evident amongst the female subjects, but this was less marked than for males.

The significant isolation and dose strength interaction is shown in Figure V(c). This reveals that, prior to drug treatment, there were significant differences in weight between the isolated animals in the three treatment conditions. Although assignment to treatment conditions was considered to be random, clearly some uncontrolled factor influenced the weights of the three groups. Possibly this was related to the relative positions of the cages of the groups in the laboratory, with consequent differences in social and/or environmental stimulation. When the lack of homogeneity of the three groups at the first weighing is taken into account, the relationship of these two variables then suggests that, although isolated animals were initially heavier than grouped animals, the effects of the two levels of IMI treatment were similar for both groups. 10mg/kg IMI was associated with weight reductions as compared to saline controls, and this was further reduced by 20mg/kg.

Figure V(a)

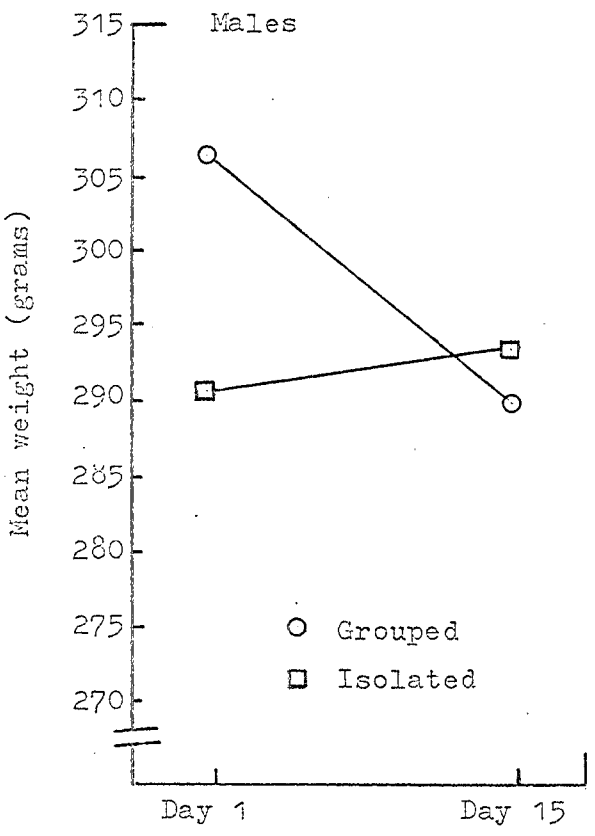
Body Weight Scores



Day 1 refers to weights obtained prior to IMI treatment, Day 15 to weights obtained on the 15th day of the chronic IMI administration. This applies on all body weight graphs.

Figure V(b)

Body Weight Scores



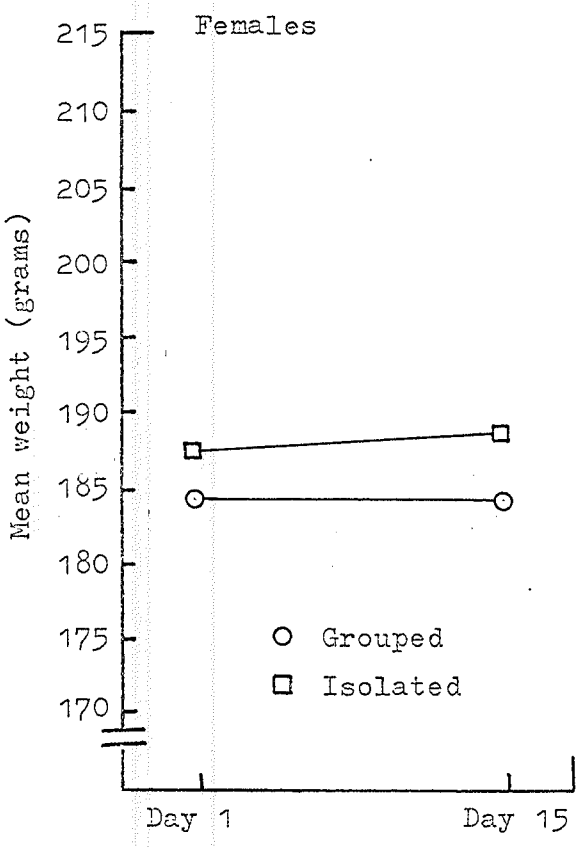
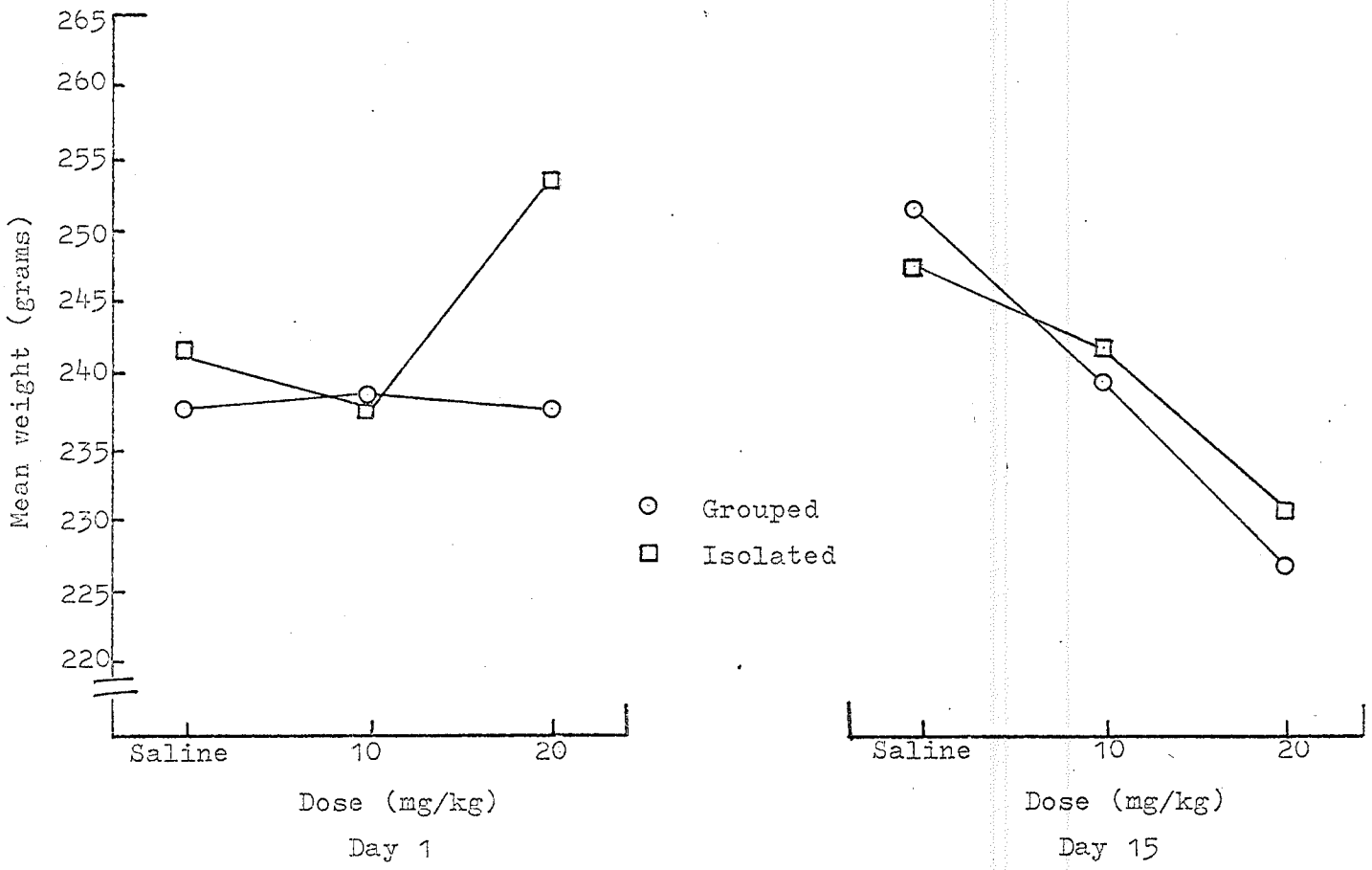


Figure V(c)

Body Weight Scores



CHAPTER 4

DISCUSSION

Preference for Novelty

The present results do not indicate a definite decrease in preference for novelty as found in previous studies using chlordiazepoxide, methylphenidate and IMI. Instead, they suggest a differential response to novelty according to the sex of the animal, the dosage level of the drug, and the time period in which the observation is made.

In terms of the mechanisms involved in the action of IMI it is difficult to account for these findings, especially that 10mg/kg decreases preferences for novelty (for example, amongst males in the third time period) as compared to saline, but that 20mg/kg may be associated with an increase in novelty preference. On the basis of a decrease in novelty preference with increasing dose strength, it had been suggested that the effect was due to the aversiveness of the novel drug state (Hughes, 1972). As with the later finding (Hughes, 1973) that LSD did not decrease novelty preference, the present results negate this hypothesis, as presumably the higher the drug dose, the more aversive the

effect, with a consequent decrease in preference for novelty.

Further support is added to the view that it is not merely the aversive effects of the novel drug state that influence responsiveness, by the fact that drug administration was carried out over a period of fourteen days, a period which should allow for at least some habituation to the drug state. This conclusion was also reached by Hughes and Greig (1975) who did not find a differential effect on this measure as a result of chronic versus acute administration of chlordiazepoxide.

Thus it seems that the effects of psychotropic drugs such as IMI on responsiveness to novel aspects of the environment are related to some "specific pharmacological action on relevant central mechanisms", rather than to non-specific factors related to psychotropic compounds in general. However the factors involved, and the precise nature of the action is still far from being understood.

The difference between the present results and those reported by Cox and Tye (1975), the only other study to the knowledge of the author in which the effects of IMI have been evaluated in relation to novelty preferences, is interesting. The major difference between the two studies seems to be the use of a single dose of IMI before testing in the latter research, as opposed to the chronic administration used in the present study. As previously discussed, differential effects

have been found when acute and chronic administration of IMI have been compared. It is possible that the decrease in novelty preference found by Cox and Tye is in part an activity-related effect, perhaps similar to the initial sedation found in humans (Guyotat, 1962; Traugott and Balanov, 1963) and the lowered activity levels found in animals. Although in rats the initial depression of activity is still found after repeated administration of IMI, it is likely, as suggested in the introduction, that the effect on motivational or affective processes develops gradually, and would thus not be evident after a single dose of IMI. Consequently it is suggested that the present results more validly reflect the effect of IMI on higher mechanisms. However, it must be pointed out that any evaluative comparison of the two studies must be tentative, because of the additional difference in testing situations, and the use of male subjects only by Cox and Tye.

Ambulation

The reduced ambulation scores under 10mg/kg and 20mg/kg of IMI after chronic treatment is consistent with a number of other findings (Furgiuele, Aument and Horovitz, 1964; Meltzer and Fox, 1971; Kulkarni and Dandiya, 1973). It seems likely that this finding is a result of the sedative properties of IMI.

The decrease in ambulation over time is also in line

with previous findings, with regard to locomotion in a novel environment in both drug- and non-drug states.

The near significant sex x isolation effect is interesting and worthy of comment because of its relation to other studies. This interaction suggested that when reared in groups, females tend to ambulate more than males, but when animals are reared in isolation this sex difference is not apparent. The higher ambulation scores amongst group-reared female rats has been reported on a number of other occasions (Archer, 1969; Hughes and Syme, 1972; Bowker, 1975). Isolation appears to have the effect of suppressing this sex difference, as do a number of other experimental manipulations such as treatment with methylphenidate (Hughes and Syme, 1972).

Rearing

Decreases in rearing responses with increasing dose strength would similarly seem to be related to the sedative properties of IMI. This is consistent with the interpretation of rearing as a general activity measure, similar in category to ambulation - i.e. as a non-specific or spontaneous motor activity rather than as an environmentally-determined exploratory activity.

The finding that females reared significantly more than males is consistent with other reports (e.g. Hughes and Syme, 1972).

Grooming

The decrease in grooming responses under both levels of IMI may also be a sedation effect. This suppression of responding is apparent only amongst isolated animals, which is understandable in view of the fact that the frequency of grooming by grouped animals in the saline condition was so low that further suppression of the response under IMI would scarcely have been possible.

It remains, however, to account for the higher frequency of grooming of isolated animals as compared to grouped animals in the saline condition. As previously mentioned, isolated animals tend to be less active in terms of locomotion and rearing responses. It is possible that these lower general activity levels are related to the higher levels of self-directed activity such as grooming, found in the present study. i.e. that amongst isolation-reared rats grooming is a substitute for the other forms of activity.

Although differences between the frequency of grooming in the three time periods were not analysed, an inspection of the raw data reveals that the highest proportion of the grooming responses occur in the second and third time periods, a finding which has previously been reported and which renders implausible the suggestion that the high frequency of grooming reflects a heightened emotionality

or "fearfulness" amongst isolated animals when placed in a novel situation (Bindra and Spinner, 1958). Presumably if this were so, a decrease in the response would occur over successive time periods, as the animal became familiar with the environment.

Thus it is difficult to account for the frequency of grooming in terms of "emotionality" concepts. This is contributed to by the general lack of understanding of the motivational factors underlying such responses in rodents. It is probably more valid to avoid explanations involving reference to such factors and accept the definition of Bolles (1960) that grooming is a form of activity that occurs after other kinds of directed behaviour have occurred. Perhaps isolated animals engage more in self-directed behaviour rather than the general activity exhibited by group-reared rats because this is a more habitual response for these animals, having had considerably less environmental stimulation than rats caged in groups.

Obviously further information is required as to the significance of the grooming response in rats and the conditions which influence it. Also the relatively low frequency of this response overall and the high intra- and inter-group variability make it probably the least informative measure in the present research. At best, when grooming is viewed as a form of "general activity" the results support the findings from the ambulation and

rearing measures that IMI depresses activity levels.

Body Weight

Although the results on this measure are somewhat complex, the general conclusion that IMI causes a reduction in weight can be drawn. This would seem to be a result of the appetite suppressant effects of IMI, reported frequently amongst human patients treated with the drug. (e.g. Azima, 1959; Dastoor, 1962).

The heavier weights amongst isolated as compared to group-reared rats reported by Morgan (1973), Bowker (1975) and others are supported in this study. Probably, as Morgan suggests, the animals reared in isolation have less to distract them from eating. The fact that isolated animals show a greater weight loss than grouped animals is probably because of their initial excess weight, which disappears rapidly when food intake is reduced. It may also be associated with a significantly greater reduction in eating. If eating is regarded in a similar way to grooming, as a self-directed behaviour, the suppression of the response under IMI thus can be seen in terms of a decrease in activity, again associated with the sedative properties of IMI.

It is not clear why the effects should not occur amongst females, but again their lower weights than males may make a reduction less likely to occur.

Significance of the Findings

The sedative properties of IMI at 10mg/kg and 20 mg/kg are clear from the depression of responses on the activity measures. Support for the proposal that the preference for novelty measure is a more sensitive indicator of the effects of IMI on CNS mechanisms comes from the different results on this measure and the activity measures. Presumably, if novelty preference were also influenced by the sedative properties of IMI, this would be shown by a consistent trend in one direction under both levels of IMI. From mere observation of the animals it appeared that possibly this measure was influenced by sedation and the general decrease in activity. In both drug treatment groups, in the latter part of the second time period and throughout the third time period, the drugged rats were seen to sit in one side of the box, exhibiting very little movement. It appeared that the side in which the rat remained motionless was based on a random choice process, rather than a true preference for novelty. However, the statistical analysis shows that, in fact, consistency in novelty preferences in the different groups was occurring, despite the "motionless" behaviour. That such preferences were apparent even when small groups of subjects were used, gives further support to the fact that preference for novelty is a sensitive measure of drug effects on motivational and/or attentional processes.

However, it is possible that a modification of the procedure could increase the validity of the findings in relation to IMI. The proposed modification would involve a longer period of time between the drug administration and testing. This is suggested because of the findings of Meltzer and Fox (1971) that the immediate effect of chronic IMI treatment was a decrease in spontaneous activity, but one day after IMI administration an increase in spontaneous activity was observed. A similar increase in activity, using rearing as the measure was reported by Kulkarni and Dandiya (1973), when observations were carried out four hours after IMI administration. Obviously further research is required to determine whether a time delay would affect the preference for novelty responses, and the exact time and also dose parameters involved.

Sex Differences

Results on preference for novelty show a clear tendency for females to be less sensitve than males to the effects of IMI. Although comparisons across species can at this stage only be speculative, this is in line with the finding amongst humans that males tend to benefit more than females from treatment with IMI. (Raskin, 1974).

In terms of the initial aims of the research it is significant that sex differences occurred on the preference for novelty measure which is presumed to measure attentional and motivational processes, and not on the activity measures. It

would be expected that the likely sex differences in biochemical functioning and endocrinological functioning believed to be involved in processes such as these, would be identified behaviourally in a test such as novelty preference, rather than on activity test. The fact that the results on ambulation and rearing were almost identical for males and females rules out the possibility of the sex differences on preference for novelty being an activity-related effect. This suggests that in future research it would be fruitless to look for sex differences on activity tests. Hoever, the sex differences obtained on novelty preference under IMI re-emphasises the need for researchers to include both male and female subjects in designs in which tests are measuring higher CNS functions. It is also clear that in the therapeutic use of IMI there is a need to control for sex differences. The precise nature of the differential responsiveness will require further research.

"Isolation - Induced Depression"

An important focus of this research has been on investigating more valid ways of evaluating antidepressant drug effects in rodent subjects. The initial hypothesis that social isolation may induce a heightened emotionality that could be used as a model of depression for the evaluation of the effects of IMI, is not strongly supported by the results. In terms of the present interpretation, isolation has altered responsiveness only on activity measures, and not on novelty preference, where, as discussed in the introduction,

emotionality effects could more validly be determined. Of course a difficulty has arisen, in that the major measures from which heightened emotionality as a result of isolation has previously been suggested, are precisely the measures that have been criticised here as lacking in validity in terms of the central porcesses involved in exploration and drug-induced states. Despite this, the fact that isolation and group rearing produce some differential effects suggests that a more thorough investigation of a developmentally-induced emotionality such as this is warranted.

Clearly larger numbers of subjects must be used and also a wider range of measures than was possible in the present study. Several factors suggest that the few tests used in this research did not adequately measure the effects produced by isolation. Firstly, it was observed that animals in the saline condition were more "jumpy", a response which intuitively at least appears to be related to emotionality levels. Secondly, as Lacey (1967) points out "situational Sterotypy" exists. This refers to the fact that the response of an "emotional" rat may differ in varying test situations. Thirdly, it is likely that individual animals will show their own particular pattern of emotional responsiveness within a single test, and to different types of tests.

Thus "emotionality" must be considered as a "complex of factors", most of which are little understood at our present

state of knowledge. As the significance of the various factors is disentangled from the present confusion, it may well be that isolation-induced emotionality will prove useful in the evaluation of antidepressant drugs and possibly psychotropic compounds in general.

Conclusions

This research confirms the initial proposal that much basic investigation at an animal level is required in evaluating the importance of pharmacological, genetic and environmental variables, before the more complex motivational processes controlling emotionality, drug responsiveness and exploration can be understood.

Several areas for future research are suggested from this study.

Firstly, further investigation of isolation as a means of inducing "depression" in animal subjects, and also of pathology produced by other forms of social and environmental manipulations is required. In fact, it is probably in the area of animal models of pathology where most work is necessary as it is essential to develop adequate models before specificity of drug action can be obtained.

Secondly, more information is required as to the variation in responsiveness under different dosage levels and durations

of administration of imipramine. Since a moderate and high dose were used here, the effects of lower doses needs to be evaluated. Use of administration periods of imipramine both below and above fourteen days would help to define more precisely the various stages of responsiveness to imipramine.

Finally, the investigation of other tests for psychotropic drug effects is necessary. Since general activity measures seem to contribute little to the understanding of drug effects, other means of evaluating effects on higher central mechanisms should be the concern of future researchers.

Appendix

Summary Tables of Analyses of Variance.

In all tables the following symbols apply:

Symbol	Factor
A	Sex
B	Isolation
C	Dose Strength
D	Time

Preference for Novelty

Source	SS	df	MS	F	
Between					
A	296.338	1	296.338		
B	79.449	1	79.449		
C	347.565	2	173.783		
AB	381.338	1	381.338	3.147	N.S.
AC	653.009	2	326.505		
BC	51.953	2	25.977		
ABC	189.51	2	94.755		
Error	7271.055	60	121.184		
Within					
D	146.287	2	73.144		
AD	4.842	2	2.421		
BD	27.62	2	13.810		
CD	129.574	4	32.394		
ACD	622.852	4	155.713	2.776	$p<.05$
BCD	270.019	4	67.505		
ABD	158.621	2	79.311		
ABCD	324.74	4	81.185		
Error	6730.112	120	56.084		

Ambulation

Source	SS	df	MS	F	
Between					
A	3.893	1	3.893		
B	1.041	1	1.041		
C	1385.861	2	629.931	48.335	p<.01
AB	55.005	1	55.005	3.837	p<.10
AC	1.676	2	0.838		
BC	1.084	2	0.542		
ABC	22.231	2	11.116		
Error	860.167	60	14.336		
Within					
D	4442.694	2	2221.347	321.934	p<.01
AD	11.454	2	5.727		
BD	2.195	2	1.098		
CD	22.778	4	5.695		
ACD	19.685	4	4.921		
BCD	14.722	4	3.681		
ABD	15.287	2	7.644		
ABCD	31.185	4	7.796		
Error	828	120	6.9		

Rearing

Source	SS	df	MS	F	
Between					
A	165.375	1	165.375	9.10	$p<.01$
B	70.041	1	70.041		
C	1801.926	2	900.963	49.58	$p<.01$
AB	4.45	1	4.450		
AC	40.111	2	20.056		
BC	7.112	2	3.556		
ABC	11.147	2	5.573		
Error	1090.389	60	18.173		
Within					
D	629.287	2	314.644	32.27	$p<.01$
AD	6.028	2	3.014		
BD	11.862	2	5.931		
CD	64.13	4	16.033		
ACD	24.111	4	6.03		
BCD	15.443	4	3.86		
ABD	25.119	2	12.56		
ABCD	27.909	4	6.98		
Error	1170.11	120	9.75		

Grooming

Source	SS	df	MS	F	
A	14.222	1	14.222		
B	99.695	2	49.848	3.43	$p<.05$
C	43.556	1	43.556		
AB	29.528	2	14.764		
AC	14.222	1	14.222		
BC	154.194	2	77.097	5.29	$p<.01$
ABC	12.694	2	6.47		
Error	873.667	60	14.561		

Body Weight

Source	SS	df	MS	F	
Between					
A	427,825.847	1	427,825.847	591.80	p<.01
B	345.34	1	345.34		
C	1454.18	2	727.09		
AB	19.507	1	19.507		
AC	2205.098	2	1102.549		
BC	780.598	2	390.299		
ABC	909.725	2	454.863		
Error	43375.25	60	722.921		
Within					
D.	126.563	1	126.563		
AD	108.507	1	108.507		
BD	423.674	1	423.674	13.36	p<.01
CD	4807.792	2	2403.896	75.80	p<.01
ACD	683.43	2	341.715	10.77	p<.01
BCD	355.93	2	177.965	5.68	p<.01
ABD	473.062	1	473.062	14.92	p<.01
ABCD	73.625	2	36.8125		
Error	1902.917	60	31.715		

TABLE A: Number of Observations out of a Total of 120 Observations of Presence in the Novel Half of the Exploration Box

		SALINE				10mg/kg IMI				20mg/kg IMI			
Time Period		1	2	3	Total	1	2	3	Total	1	2	3	Total
Grouped	Male	26	34	27	87	25	23	35	83	24	33	21	78
		22	30	19	71	20	11	1	32	20	35	40	95
		22	22	17	61	21	9	0	30	0	33	11	44
		24	30	30	84	25	20	5	50	14	27	37	78
		27	34	14	75	30	14	26	70	16	9	1	26
		29	23	35	87	23	22	18	64	23	4	37	64
	Female	21	30	29	80	23	29	30	82	22	35	27	84
		18	31	27	76	28	27	22	77	27	27	40	94
		27	33	29	89	30	23	33	86	26	33	25	84
		32	23	27	82	15	31	40	86	27	23	37	87
		22	21	32	75	26	23	33	82	24	29	25	78
		17	30	35	82	26	25	5	56	28	26	15	69
Isolated	Male	22	22	32	76	22	33	29	84	21	17	28	66
		22	24	29	75	12	5	0	17	35	36	40	111
		26	29	34	89	22	34	24	80	20	17	0	37
		28	28	28	84	17	24	4	45	19	39	37	95
		23	29	25	77	23	19	34	76	24	25	30	79
		24	22	20	66	18	23	1	42	18	11	29	58
	Female	31	26	28	85	22	29	40	91	24	26	14	64
		28	31	33	92	30	18	33	81	19	35	35	89
		19	25	12	56	21	24	13	58	28	40	15	83
		10	9	0	19	25	19	39	83	20	24	0	44
		23	28	32	83	22	30	36	88	24	18	19	61
		29	27	18	74	22	20	17	59	21	9	0	30

TABLE B: Number of Cells Entered During the 10 minute Observation Period in the Exploration Box

		SALINE				10mg/kg IMI				20 mg/kg IMI			
Time Period		1	2	3	Total	1	2	3	Total	1	2	3	Total
Grouped	Male	16	9	6	31	17	7	1	25	15	2	5	22
		19	11	7	37	19	6	3	28	8	4	0	12
		20	11	7	38	17	3	0	20	0	11	3	14
		18	10	8	36	20	10	2	32	11	12	4	27
		18	14	8	40	13	4	5	22	15	5	1	21
		20	11	7	38	9	5	7	21	17	2	3	22
	Female	23	12	12	47	12	8	6	26	17	9	4	30
		24	14	10	48	17	8	9	34	14	4	1	19
		19	9	9	37	16	7	1	24	11	2	5	18
		18	7	7	32	11	8	0	19	18	12	4	34
		25	13	13	51	16	7	7	30	19	4	7	30
		19	15	9	43	14	3	1	18	11	3	1	15
Isolated	Male	17	9	9	35	15	9	7	31	11	5	7	23
		15	11	9	35	12	6	0	18	10	5	2	17
		21	10	5	36	15	7	13	35	18	8	0	26
		22	12	10	44	11	6	2	19	15	3	5	23
		25	13	14	52	16	8	3	27	14	8	6	28
		17	13	12	42	12	6	1	19	14	5	4	23
	Female	23	10	10	43	13	6	1	20	10	4	6	20
		21	8	6	35	14	7	3	24	13	2	4	19
		18	10	8	36	14	10	3	27	12	8	1	21
		11	4	0	15	12	8	2	22	15	7	3	25
		28	17	10	55	16	9	5	30	13	5	0	18
		19	11	8	38	16	5	3	24	13	5	3	21

TABLE C: Number of Rearing Responses Observed

Out of a Total of 120 Observations

		SALINE				10mg/kg IMI				20mg/kg IMI			
Time Periods		1	2	3	Total	1	2	3	Total	1	2	3	Total
Grouped	Male	9	9	5	23	13	9	0	22	7	0	2	9
		9	11	6	26	5	11	6	22	7	6	0	13
		12	10	6	28	3	0	0	3	0	3	0	3
		14	15	10	39	11	9	0	20	6	15	1	22
		15	7	10	32	12	0	11	23	3	0	0	3
		11	15	6	32	7	0	0	7	13	2	4	19
	Female	18	14	16	48	13	7	10	30	8	3	1	12
		13	15	10	38	13	8	11	32	4	0	1	5
		12	9	15	36	12	5	0	17	8	0	2	10
		15	7	13	35	5	8	0	13	10	5	2	17
		12	17	14	43	12	10	18	40	11	4	3	18
		10	17	5	32	10	6	1	17	10	2	1	13
Isolated	Male	11	15	7	33	9	2	5	16	3	1	0	4
		9	6	2	17	7	4	0	11	6	2	0	8
		9	4	9	22	7	9	8	24	8	1	1	10
		13	7	7	27	2	5	0	7	4	0	0	4
		15	13	7	35	8	6	1	15	5	9	5	19
		11	12	10	33	5	3	0	8	5	0	2	7
	Female	10	16	3	31	7	5	3	15	5	4	2	11
		9	10	10	29	9	8	6	23	4	0	3	7
		13	14	15	42	11	9	0	20	2	2	6	10
		7	5	0	12	12	9	4	25	5	6	0	11
		10	14	13	37	9	4	6	19	14	10	2	26
		15	16	6	37	10	5	7	22	1	1	0	2

TABLE D: Number of Grooming Responses Observed During the 10 minute Observation Period

	Grouped			Isolated		
	Saline	10mg/kg	20mg/kg	Saline	10mg/kg	20mg/kg
Male	3	0	7	2	0	0
	3	15	7	3	11	2
	4	0	5	17	1	0
	0	2	3	10	10	0
	1	0	3	10	0	4
	1	6	6	2	4	2
Female	2	2	2	7	3	1
	1	0	3	23	6	2
	6	1	1	6	1	1
	1	3	1	3	4	2
	5	0	2	7	4	0
	4	0	0	2	5	1

TABLE E: Body Weight in Grams on the First and Fifteenth Days of Chronic IMI Administration

		Saline		10mg/kg IMI		20mg/kg IMI	
		Day 1	Day 15	Day 1	Day 15	Day 1	Day 15
Grouped	Male	290	309	326	330	323	292
		289	311	257	267	308	260
		285	310	285	277	275	260
		275	296	290	297	315	280
		295	317	306	318	222	287
		305	326	281	282	310	280
	Female	200	200	186	192	180	171
		193	202	184	181	189	175
		187	196	185	186	186	178
		184	186	194	191	180	179
		169	176	168	173	183	176
		180	191	196	183	178	180
Isolated	Male	305	306	300	327	254	217
		314	309	277	277	326	290
		268	262	278	280	313	282
		362	363	330	316	344	303
		287	289	250	253	297	267
		306	324	305	296	317	280
	Female	180	204	171	172	200	185
		193	186	179	192	200	187
		188	179	165	172	182	174
		158	166	194	198	205	189
		161	177	200	197	204	190
		175	196	202	209	204	189

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